

A computational high-throughput screening approach of iNKT-agonists: a novel tool to find optimized iNKT cell ligands.

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Introduction

Depending on the environment and the activating glycolipids, **iNKT cells** are known to induce **T-helper 1 and/or T-helper 2** cytokines. This highly versatile nature makes these innate-like cells very interesting targets for **immunomodulation**. The design of iNKT cell ligands with selective Th1 and Th2 properties requires refined structural insights. Therefore, the **chemical space** of 333 currently known iNKT-activators, including several newly tested analogs, was visualized by chemical descriptors which were calculated for each individual analog. The **immunological space** consisted of cytokines in different test-systems. With these two information-sets, **structure-activity models** were developed using a system biology computational approach. We present highly sensitive and specific predictive models that can be further exploited for **in silico screening of potential glycolipids**, thereby reducing the attrition rate.

Dataset

- ✓ **Web of Science**, keywords 'α-GalCer-analogs' and 'iNKT cell activators'
- ✓ Period: **till the end of 2012**
- ✓ Articles containing defined chemical structures accompanied with quantitative cytokine-values + new analogs from our research group => **333 glycolipids**
- ✓ Minimal energy **3D optimization** of chemical structures using Hyperchem software
- ✓ All of the immunological responses were **normalized to the response of α-GalCer**
- ✓ The immunological space consists of the cytokines **IL-2, IFN-γ, IL-4 and IL-13** in **five test-systems**: *mice/in vivo*, *mice/in vitro/cell-cell*, *mice/in vitro/cell-plate*, *human/in vitro/cell-cell* and *human/in vitro/cell-plate*

Chemical space

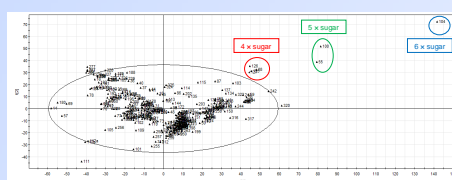
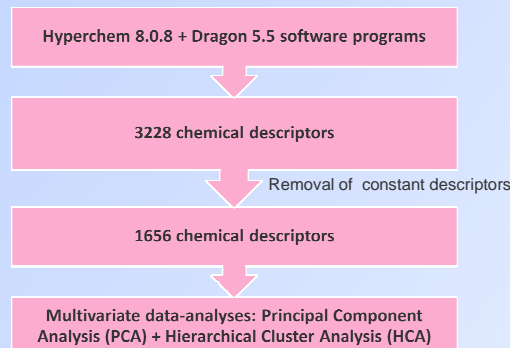


Figure 1. PCA score plot with the two first principal component vectors t(1) and t(2). Each PCA vector represents a specific combination of the 1656 chemical descriptors.

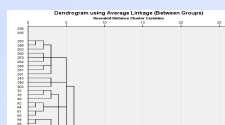


Figure 2. An illustration of the physico-chemical hierarchical cluster analysis (HCA).

Immunological space

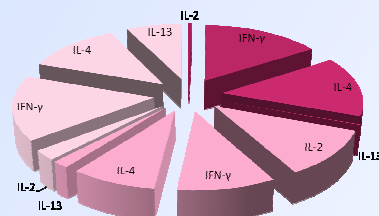


Figure 3. The immunological space in literature for the 3 most important test-systems: *mice/in vivo* (■), *mice/in vitro* (■) and *human/in vitro* (■).

Before modelling, the immunological responses were transformed in dimensionless **desirability (D)** values, normalized combinations of desired cytokine-values (d). For example, an analog with high IFN-γ and low IL-4 values has a high Th1 D-value.

$$d(Y) = \frac{0.9-0.1}{Y_{max}-Y_{min}} \times (Y_i - Y_{min}) + 0.1$$

for parameters to be maximized

$$d(Y) = \frac{0.1-0.9}{Y_{max}-Y_{min}} \times (Y_i - Y_{min}) + 0.9$$

for parameters to be minimized

$$D = \sqrt[n]{\prod_{i=1}^n d_i^2}$$

Structure-immune modelling

- ✓ Chemical space + immunological space => **structure-immune models** were computed
- ✓ Goodness-of-fit R² values + predictive Q² values (table 1) => **our models well explain the variability observed**
- ✓ In our laboratory, we **analyzed 16 new analogs** using *mice/in vivo* experiments and found only compounds 2 and 3 to have a higher Th1 desirability than α-GalCer 1
- ✓ Our computed model also indicated only these two analogs as more desirable than α-GalCer 1, which exemplifies the good R² and Q²

Table 1. The explained variability of the models.

| Functionality | R ² | Q ² |
|--|----------------|----------------|
| Th1 <i>mice/in vivo</i> | 0.941 | 0.742 |
| Th2 <i>mice/in vivo</i> | 0.962 | 0.783 |
| Th1 <i>human/in vitro</i> <i>mice/in vitro</i> | 0.722 | 0.512 |
| Th2 <i>human/in vitro</i> <i>mice/in vitro</i> | 0.640 | 0.512 |

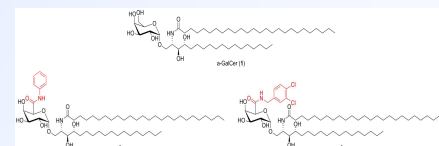


Figure 4. α-GalCer (1) with two novel strong Th1 analogs.

Conclusion

The **in silico** analysis combining the chemical and immunological space of iNKT activators as described here, provides a novel tool in predicting the functional impact of altered iNKT agonists, decreasing analysis time and costs for functionality analysis.

References

An *in silico* approach for modelling Th polarizing iNKT cell agonists. Anton De Spiegeleer, Evelien Wynendaele, Matthias Vandekerckhove, Sofie Stalmans, Maxime Boucart, Nele Van Den Noortgate, Serge Van Calenbergh, Sandrine Aspeslagh, Koen Venken and Dirk Elewaut (2013) Manuscript in preparation.